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CORRELATION BETWEEN CHEMICAL CHARACTERISTICS AND BIOLOGICAL REACTIVITY OF PARTICULATE MATTER IN AMBIENT AIR

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CORRELATION BETWEEN CHEMICAL CHARACTERISTICS AND BIOLOGICAL REACTIVITY OF PARTICULATE MATTER IN AMBIENT AIR

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Science and Technology Branch
Environmental Sciences Division
Alberta Environment

April 2001

Pub. No: T/602
ISBN: 1709-8 (Printed Edition)
ISBN: 1710-1 (On-Line Edition)
Web Site: http://www.gov.ab.ca/env/

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FOREWARD

The release and accumulation of particulate matter in ambient air have been linked to a number of human health effects, including mortality. However, the biological mechanisms by which particulate matter affects human health is less clear. Some hypotheses suggest that the health effects of particulate matter are due to its physical and chemical properties.

There are two classes of particulate matter that are most extensively studied - particulate matter less than 10 micron in diameter (PM_{10}) and particulate matter less than 2.5 micron in diameter ($PM_{2.5}$). $PM_{2.5}$ is also called the "fine fraction" of particulate matter. It is thought to be more detrimental to human health because of its ability to penetrate deeper into the cardiovascular system, its high surface area, and its associated chemical species.

Ambient particulate matter can be attributed to a variety of point and area sources such as coal and wood burning, oil and gas processing and flaring, road dust and transportation, and forest fires. A significant portion of fine particles is also formed in ambient air due to the presence of precursors such as sulphur dioxide, nitrogen oxides, ammonia, and hydrocarbons. Those particles formed in the ambient air are called secondary particulates. The chemical composition of ambient particulate matter could be drastically different because of their different origin.

The Air Research Users Group of Alberta Environment commissioned this literature review on the correlation between chemical characteristics and biological reactivity of particulate matter in ambient air. The information provided in this review will help Alberta Environment develop research programs in this important area.

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SUMMARY

The effect of particulate matter on human health is currently an important environmental concern. Considerable evidence of health effects provided by epidemiological studies has resulted in regulation of atmospheric particulate levels in Canada and other countries. Despite this data, a definitive biological mechanism has not been identified. Furthermore, ambient particles possess a complex and variable chemical profile, and the most important components have not been positively determined. These uncertainties have resulted in criticism of the standards proposed by environmental agencies.

To better understand the effects of particulate matter, research has been conducted using animal, cellular, and human exposure models. Biological effects being studied include inflammatory responses, genotoxicity, and cardiovascular effects. Several theories have been developed to relate the results found in laboratory studies to the effects observed in the epidemiological surveys. In addition, many groups have attempted to identify the most important chemical components of the particulate samples being used. The most commonly studied chemicals are reviewed in this report, and results from recent experiments investigating their relative importance are included. As well, a summary of methods used in particulate matter research is provided, including collection and characterization of particle samples, treatment of cells, and common biological responses. This is followed by a list of equipment and expertise that may be required to conduct research in this area.

The available literature on the biological effects of particulate matter does not indicate a clear mechanism of action responsible for the health effects observed in exposed human populations. Further research is required to identify the most important effects and the chemical components involved in these responses. This goal is complicated by the complex and changing nature of airborne particles, depending on local pollution sources, daily and seasonal variations, and climate. Future studies should account for this variation by using a greater number of particle samples from different sources. The use of animal models for testing large numbers of samples is not feasible due to cost and time requirements, therefore a reliable and inexpensive cellular assay would be desirable for screening purposes. Additional information on the biological mechanisms of particle toxicity would strengthen the associations observed in epidemiological studies.

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1.0 INTRODUCTION

Particulate airborne matter and its effects on human health is an issue that has received considerable attention over the past several years. Concerns over this form of pollution resulted in new air quality standards for PM_{10} (particulate matter with an aerodynamic diameter below 10 µm) being introduced by the US EPA in 1987. In 1997 more stringent standards were proposed based on a even smaller fraction, $PM_{2.5}$ (less than 2.5µm), but their implementation was blocked by a lawsuit from industry groups in 1999 (Ware 2000). The major factor behind these revised standards was the substantial body of epidemiological evidence associating fine particulate matter (PM) with adverse health outcomes in exposed human populations. These associated outcomes have included: excess deaths from cardiopulmonary disorders and lung cancer, increased hospitalization rates for respiratory disorders, increased respiratory symptoms and emergency visits for people with asthma and chronic obstructive pulmonary disease (COPD), and reduced lung function (reviewed in Pope 2000). Despite this evidence, questions about the analytical methods used in the epidemiological studies and the lack of a plausible biological mechanism for the observed effects have been raised. These uncertainties have resulted in criticism of the air quality standards proposed by the EPA (Smith et al. 1999, Gamble 1998, Kaiser 1997).

In 1997, a report outlining the National Ambient Air Quality Objectives for Particulate Matter in Canada was prepared by the CEPA/FPAC (Canadian Environmental Protection Act / Federal-Provincial Advisory Committee) Working Group on Air Quality Objectives and Guidelines. A revised version of this document (CEPA/FPAC Working Group 1999) was used by the Canadian Council of Ministers of the Environment (CCME) in June 2000 to establish the Canada-Wide Standard (CWS) for PM. The risk characterization section of the science assessment in this report recognizes that uncertainties in a causal relationship include: "lack of an accepted biologically demonstrated mechanism; lack of quantitative support from experimental animal and/or human clinical studies; difficulty in discerning what, exactly, is the toxic moiety…". It also suggests that: "further research, both *in vitro* and *in vivo*, is needed on the basic mechanism(s) of toxicity of particles to elucidate a biologically plausible mechanism of action of particles. This need is critical in view of the provisional nature of conclusions on causality from the indirect evidence provided by the ecological epidemiology data set". For these reasons, significant research efforts are being carried out in Canada and other countries to further understand the relationship between PM and its biological effects. Factors being considered in the assessment include the physical characteristics of ambient particles, most importantly their size, as well as their chemical composition. Identification of the most important components that induce a biological response would strengthen the association between PM and adverse health effects demonstrated in the epidemiological studies.

Considerable research has been undertaken on the biological effects of particulate matter (reviewed in CEPA/FPAC Working Group 1999, Casillas et al. 1999, Salvi and Holgate 1999, Blomberg 2000, Dreher 2000, Pope 2000). Experimental approaches have included *in vivo* animal toxicology studies, *in vitro* studies using a variety of cell types from different species, controlled human exposure trials, and re-analysis of available epidemiological data combined with information on physical and chemical characteristics of ambient PM from the associated areas. Laboratory experiments have utilized a variety of defined particles, including surrogate synthetic particles of known size and composition. Several studies have used particles from ambient air or isolated pollution sources, and have attempted to assess the contribution of different chemical fractions of PM to the effects observed in the biological system being monitored. The types of cellular or biochemical responses being tested in either *in vivo* or *in vitro* systems are quite diverse, but most are related to either pulmonary or cardiovascular effects observed in the epidemiological studies.

The purpose of this document is to provide a review of the major research findings in this area with emphasis on studies assessing the effect of chemical components of fine particulate matter on biological responses. A summary of expertise and equipment required for PM research, as well as some experimental procedures currently used in this area, are also presented.

2.0 LITERATURE REVIEW

2.1 Chemical Composition of Particulate Matter

Airborne particulate matter consists of a complex mixture of particles of varying size and chemical composition. Several reports have been published regarding the physical and chemical characteristics of PM sampled from a variety of locations (summarized in CEPA/FPAC Working Group 1999). The chemistry of ambient particles is dependent on a number of factors, including particle size and sources of particulate pollution in the local environment. Despite the diversity of properties in different PM samples, a number of constituents have been identified including elemental carbon, various organic carbon compounds, trace metals, sulfate and nitrate aerosols, and biological material.

Elemental carbon particles have been suggested as possible mediators of biological responses to particulate matter. Carbon particles are typically very small, less than 1 μm in diameter, which greatly enhances their penetration into the deeper regions of the lung instead of being filtered out by the upper respiratory tract. Furthermore, these particles may act as carriers for other toxic compounds that are commonly found adsorbed to the surface of carbon particles, thereby increasing their toxicity in the lung.

A wide range of organic compounds have been identified in PM_{10} samples (CEPA/FPAC Working Group 1999). Major classes of these compounds include alkanes, alkenes, polycyclic aromatic hydrocarbons (PAHs), oxidized hydrocarbons including acids, aldehydes, ketones, quinones, phenols and esters, and nitro- and sulfur-substituted organic compounds. Many of these chemicals have been tested for carcinogenic and non-carcinogenic effects in toxicology studies, and are potential agents in the toxicity of ambient particulate matter. PAHs are probably the most extensively studied class of organic pollutants, and many have been found to possess mutagenic and carcinogenic potential. PAHs and other organic compounds are generally found in the fine particle fraction ($PM_{2.5}$) and are a major component of diesel exhaust particles, an important source of $PM_{2.5}$ in urban environments around the world (Salvi et al. 2000).

Trace levels of several metals are typically found in emissions from industrial processes including smelting and power generation. The chemical profiles of these particulate emissions can vary greatly depending on the source, with some elements being unique to a given industry. Several metals have been suggested as potential agents in the biological response to PM, including iron, copper, vanadium, zinc, and nickel. Possible mechanisms of toxicity for these first-row transition metals will be discussed later. Trace metals are typically found in the $PM_{2.5}$ fraction, adsorbed to the surface of carbon or other particles. Other elements of crustal origin are found in coarse particulate matter, but are not considered likely to be a major source of particle toxicity.

Nitrate and sulfate particles are formed by secondary chemical reactions in the atmosphere. Common formation processes were summarized in the CEPA/FPAC Science Assessment Document (CEPA/FPAC Working Group 1999). These compounds are also associated with the fine particulate fraction (<2.5 μm). Their contribution to the respiratory effects of PM may be a result of the inherent acidity of particles containing nitrates and sulfates.

Other components of airborne particulate matter that have been proposed to be responsible for respiratory health effects are compounds of biological origin. One example is pollen grains, which are found in the coarse fraction of PM and can induce a range of allergic and inflammatory responses in susceptible individuals. Of additional concern are endotoxins, or components of bacterial cell walls, which are associated with fine particles and may also induce biological responses.

Due to the complex and variable nature of ambient particulate matter, it is difficult to characterize the agents that may be responsible for the health effects observed in epidemiological studies. This objective is further complicated by the reality that PM samples exhibit geographical, source, and short- and long-term temporal variation in chemical characteristics. Nevertheless, research efforts have identified links between several components of PM and biochemical or molecular responses observed in model systems.

2.2 Biological Effects of Particulate Matter

Research on the biological effects of particulate matter has involved a number of different approaches. Earlier studies on mechanisms of toxicity involved exposure of rats or other animals to well-characterized particles, typically by direct application to the respiratory tissues. Health endpoints that were evaluated included lung function, host defense systems, tissue cellular and biochemical responses, mortality, cardiovascular response, effects on compromised lungs, and cancer. An extensive review of these toxicological experiments was presented in the National Ambient Air Quality Objectives for Particulate Matter, Science Assessment Document (CEPA/FPAC Working Group 1999). Observed effects in these animal studies included: decreased lung function; decreased particle clearance from the lung (some studies showed an increase); increased alveolar macrophage numbers and activity (except in cases of high particle cytotoxicity); biochemical changes associated with activation of alveolar macrophages (including increased cytokine production); decreased antimicrobial defense capacity; morphological changes in lung tissue; cardiovascular abnormalities; and lung cancer (when exposed to high levels of particles). While these studies demonstrated biological responses to particulate matter, a number of questions were raised concerning the cause of particle toxicity. These included the role of size and chemical composition of particles in the toxic response, as well as the contribution of "particle overload" to the observed effects.

Research has shown that smaller particulate matter is the most damaging to human health (CEPA/FPAC Working Group 1999, Salvi and Holgate 1999). Initially PM_{10}, corresponding to particles with an aerodynamic diameter of less than 10 μm, drew attention. Recent studies have found biological effects to be even more pronounced for particles less than 2.5 μm ($PM_{2.5}$), commonly referred to in the literature as the fine fraction of particulate matter. The importance of $PM_{2.5}$ has been demonstrated recently in laboratory experiments as well as epidemiological studies (Dreher 2000, Hsiao et al. 2000, Pope 2000, Schwartz et al. 1996). The reason for the enhanced effects of smaller particles is suggested to be three-fold. First, an aerodynamic size of less than 10 μm allows these particles to be inhaled deeply into the lungs, where they may exert their toxic effects on alveolar cells (macrophages, neutrophils and epithelial cells). This deposition is greatest for the $PM_{2.5}$ fraction (Salvi and Holgate 1999). Secondly, as particle size decreases, the ratio of surface area to volume (mass) of the particle increases. This occurrence is based on the mathematical equations for surface area ($S.A.= 4\pi r^2$) and volume ($V= {}^4/_3\pi r^3$) of a sphere, which may be used to approximate the properties of particulate matter. A 10-fold decrease in the radius of a particle would consequently result in a 10-fold increase in the surface area to mass ratio. This substantial increase in surface area for a given particle mass allows greater adsorption of chemical constituents onto the particle surface and increases the area of contact with target cells (Salvi and Holgate 1999). Finally, the chemicals most often associated with PM effects (organic compounds, trace metals, sulfates and nitrates) are found predominantly in the fine fraction of ambient particles (CEPA/FPAC Working Group 1999). Consequently, research conducted more recently on mechanisms of toxicity is focusing on the PM_{10}, or more importantly $PM_{2.5}$, fractions of ambient particulate matter.

In addition to particle size, the animal studies reviewed in the Science Assessment Document attempted to determine the chemical constituents in particulate matter that were responsible for the observed toxic effects (CEPA/FPAC Working Group 1999). Generally speaking, these studies were unable to positively identify the most important chemical species. Acidic aerosols including sulfates and nitrates received considerable attention as a possible agent involved in inflammation and other respiratory symptoms. A recent review on chemical constituents and toxicity of PM suggested that acidic compounds alone are not likely to cause the health effects observed in epidemiological studies, but that particle acidity may enhance the action of other components (Dreher 2000). An additional suggestion was that iron (Fe^{3+}) or other first-row transition metals adsorbed on the particle surface induced an inflammatory response by generating higher levels of reactive oxygen species (ROS) in the lung. It was also proposed that ROS generation may be responsible for the observed cardiovascular effects. Lung cancer studies concentrated on the diverse range of organic compounds that have been found adsorbed to the surface of particles. These contaminants include PAHs, a class of compounds containing several mutagenic and carcinogenic species. Although extremely high levels of exposure to these PAH-containing particles were necessary to induce formation of lung tumors in animals, concern remains about the effects of chronic human exposure to ambient levels of these compounds.

The issue of how to extrapolate results from high levels of exposure in animal toxicology studies to low levels which are relevant to human exposure situations is an ongoing concern for environmental contaminant research, and particulate matter is no exception. For all the effects described above, animals were exposed to high doses of particles so that biological responses were likely to be observed. Uncertainties related to high levels of exposure include whether or not there are different mechanisms of toxicity at high versus low doses, and whether there is a threshold concentration of a substance below which its effects are no longer observed. While methods for dealing with these uncertainties have been developed, there is an additional consideration related to particulate matter. For many of these studies, the dosages of particulate matter used were high enough to exceed the capacity of the mechanisms responsible for clearing particles from the lungs, resulting in "particle overload". The resulting increase in residence time of the particles in the lung has been suggested to be at least partly responsible for many of the biological effects demonstrated in animal studies (CEPA/FPAC Working Group 1999). Consequently, there is a need to conduct research with smaller doses which represent PM levels in ambient air in order to clarify related biological impacts and associated mechanisms.

More recent experimental approaches to determine the numerous biological effects of particulate matter have included *in vitro* studies, *in vivo* animal experiments, and controlled human exposure studies (Dreher 2000, Blomberg 2000). Increased morbidity and mortality resulting from inflammatory responses in the lung has been associated with exposure of human populations to particulate matter. This is particularly important for the elderly, infants, and individuals with pre-existing inflammatory diseases such as bronchitis, asthma and pneumonia (Pope 2000). Consequently, the majority of research on PM has focused on the inflammatory response in lung tissues and cells. This response is characterized by increased numbers of polymorphonuclear (PMN) cells (alveolar macrophages and neutrophils) in the lungs, increased production of inflammatory mediators including cytokines and nitric oxide, and morphological pulmonary injury. Some recent publications have reviewed inflammatory effects in various experimental systems (Blomberg 2000, Salvi and Holgate 1999). The involvement of transition metals and reactive oxygen species in these biological responses continues to be a common theory. Reviews on mechanisms of ROS toxicity have also been published (Casillas et al. 1999, Martin et al. 1997).

Compounds of biological origin have also been associated with PM-induced inflammatory effects. Of these, endotoxins (lipopolysaccharides from the cell wall of gram negative bacteria) are the most important mediator found in fine particulate matter and have been studied by several groups. Other research groups have investigated the involvement of polycyclic aromatic hydrocarbons adsorbed to the surface of diesel exhaust particles in allergic responses to PM. The suggested mechanism involves an increase in production of immunoglobulin E (IgE), and has also been recently reviewed (Diaz-Sanchez 1997).

This report will supplement these reviews by concentrating on more recent findings related to inflammatory effects of PM, particularly the contributions of different chemical components. In addition to the inflammatory response, studies investigating the cardiovascular effects and genotoxicity of PM have been published. Genotoxicity is associated with the possibility of an increased risk of developing lung cancer, which has been suggested in some epidemiological studies (Dockery et al. 1993). Some examples of research in these areas will be included in this section.

2.2.1 Inflammatory Responses Induced by PM

Most of the health effects of PM demonstrated in epidemiological studies relate to the exacerbation of respiratory symptoms in susceptible individuals. The ability of transition metals and other chemical species to induce oxidative stress or generate reactive oxygen species (ROS) in the lungs is commonly suggested as a mechanism of particulate matter toxicity (Dreher 2000, Martin et al. 1997). Li et al. (1997) used *in vivo* and *in vitro* models to investigate the effects of ambient PM_{10}. The *in vivo* system consisted of rats treated with PM from which lung macrophages were recovered by bronchoalveolar lavage (BAL). A human lung carcinoma cell line (A549) was used as a surrogate for lung epithelial cells for *in vitro* exposure studies. In both cases, an inflammatory response characterized by increased levels of tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) production was observed. Epithelial cell injury was also indicated by an increase in cell membrane permeability in both systems. These responses were suggested to be a result of the oxidant activity of the PM_{10} samples, since a decrease in the lung antioxidant glutathione (GSH) was also observed.

Recent studies have attempted to determine the components of ambient PM that are responsible for these inflammatory responses. Ambient total suspended particulates from Provo, Utah were tested for biological reactivity using *in vivo* (rat) and *in vitro* (human) systems (Kennedy et al. 1998). The particles were well characterized, with metals, sulfate, and endotoxin content being measured. The overall ability of the particles to generate reactive oxygen species was also determined. These particles were found to induce an inflammatory response *in vivo*, characterized by an increase in polymorphonuclear cells in the bronchoalveolar lavage fluid.

An increase in production of cytokines [e.g., interleukin-6 (IL-6) and IL-8] as well as activation of the nuclear transcription factor NF-κB was also demonstrated *in vitro*. NF-κB is an important mediator of the inflammatory response. Upon activation through stimuli including oxidants, this DNA-binding protein migrates from its normal position in the cytosol into the nucleus where it activates transcription of several inflammatory genes including the genes for cytokines IL-2, IL-6, IL-8, and TNF-α (Abbas et al. 1994, Carter et al. 1997, Jimenez et al. 2000). The observed responses were found to be correlated with high levels of copper in the particles, but not with iron, lead or zinc also present in the sample. It was also determined that the soluble fraction of the particles, or the content adsorbed to the particle surface that was removed with agitation in a buffer solution, was more important than the insoluble fraction.

Other research groups have also assessed the involvement of metals in the toxicity of particulate matter from various locations. Carter et al. (1997) used an *in vitro* exposure of normal human bronchial epithelial cells to residual oil fly ash (ROFA). ROFA is a source-specific particle, emitted by industries that burn heavy oil, which contains several transition metals but only low levels of organic or biological components. They demonstrated increased expression of mRNA encoding the cytokines IL-6, IL-8 and TNF-α, as well as increased levels of these proteins in supernatant from epithelial cells exposed for 2 hr. These responses were inhibited by deferoxamine, a transition metal chelator, and dimethylthiourea, a free radical scavenger. This supported the mechanism of transition metal-induced free radical formation and possible involvement of NF-κB transcription factor. They proposed that vanadium present in ROFA, and not iron or nickel, was responsible for the increased cytokine production.

More recently, the same group was involved in a study of ambient PM_{10} from the Utah Valley (Frampton et al. 1999). Particulate samples collected in three consecutive years differed in that the main pollution source, a steel mill, was closed during the second year. Year 2 ambient PM_{10} contained lower levels of iron, copper and zinc, and had a lower oxidant generating potential than years 1 and 3 when the mill was operational. *In vitro* exposure of human BEAS-2B respiratory epithelial cells to extracts from the PM_{10} filters resulted in increased production of IL-6 and IL-8 for years 1 and 3, but not during the closure period in year 2. Cytokine secretion was again inhibited by deferoxamine and dimethylthiourea. This suggested a causal relationship of transition metals in the inflammatory response, although it was not clear which of the metals (iron, copper, zinc) was the most important factor. Nevertheless, these results were significant since they were supported by epidemiological data showing an improvement in several health indicators in the resident population during the closure.

Other groups have provided evidence that iron is the most active species in the oxidative stress response. A549 cells were exposed *in vitro* to ambient PM_{10} collected in London and Edinburgh, and the nuclear translocation, DNA-binding, and transcriptional activation of NF-κB was monitored (Jimenez et al. 2000). An increase in the activity of NF-κB was observed after exposure to total PM_{10} as well as the soluble fraction. Furthermore, pretreatment of PM samples with a combination of two metal chelators, deferoxamine and ferrozine (specific for Fe^{2+}) decreased the effects. This suggested that iron was a key component in the observed activation of NF-κB by these ambient particles. A similar result was described for PM_{10} samples collected during spring and winter in Finland (Salonen et al. 2000). This study raised the issue of seasonal variations in the chemical composition of PM, indicating that testing of ambient particle toxicity should account for this variation. Both samples induced nitric oxide production in a mouse macrophage cell line, whereas only the spring sample caused increased production of IL-6. Chemical analysis of the samples was performed using ion chromatography and inductively coupled plasma mass spectrometry (ICP-MS). The most noticeable difference between the two samples was a 10X higher concentration of iron in the spring sample. This supported the association of iron with increased cytokine expression, however the researchers were unable to rule out the involvement of other components including endotoxin.

Components of particulate matter other than transition metals have also been associated with inflammatory responses in exposure models. Imrich et al. (2000) treated rat BAL alveolar macrophages *in vitro* with ambient $PM_{2.5}$ from Boston air for which trace element and endotoxin levels were determined. They monitored release of cytokines TNF-α and macrophage inflammatory protein (MIP-2) as well as nitric oxide. A response to both the soluble and insoluble fractions of PM was observed, but with the insoluble fraction having a greater effect. Variability in both the soluble/insoluble ratio and potency of PM collected on different days was found to correlate. This suggested that the different PM samples varied in the source profile generating the particles. Interestingly, the observed responses did not significantly correlate with any element in the insoluble fractions as determined by ICP-MS, including copper and iron. However, endotoxin adsorbed to the particle was shown to be responsible for the inflammatory effects. This role of endotoxin in the effects of PM was investigated further by determining the levels of endotoxin in the soluble and insoluble fractions, and determining the influence of these PM samples on BAL macrophage phagocytosis of particles as well as TNF-α and MIP-2 production (Ning et al. 2000). Both biological activity and endotoxin content were found to be higher in the insoluble fraction, and the involvement of endotoxin in the response was confirmed by inhibition studies. However, only a portion of the inflammatory response was attributable to endotoxin, suggesting that other components in PM were partly responsible. In other studies, endotoxin was determined to have little or no involvement in the observed *in vitro* responses to ambient PM_{10} samples (Jimenez et al. 2000, Frampton et al. 1999). These inconsistent results on the importance of endotoxin may be explained by the variability of PM samples being used. Whatever the reason, further research is necessary to clarify its involvement in particulate matter toxicity.

A recent publication assessed the effects of ambient $PM_{2.5}$ from Vermont and ultrafine carbon black particles (< 0.1 μm diameter) on a mouse alveolar epithelial cell line (Shukla et al. 2000). Both particle types induced oxidant production in the cells as well as increased NF-κB activity. Pretreatment of the $PM_{2.5}$ sample with deferoxamine did not reduce the observed effects, suggesting that iron present in the particles was not responsible for the generation of reactive oxygen species (ROS) in the cells. This theory was supported by the observed effects of iron-free ultrafine carbon black particles. The conclusion was reached that alternative pathways of ROS generation may be involved in activation of NF-κB, and that the ultrafine fraction of $PM_{2.5}$ may be an important factor.

Yet another component of PM that has been implicated in the inflammatory response is the group of organic compounds. Diesel exhaust particles (DEPs) contain many different organic species and have been found to increase IgE production in animal studies (Diaz-Sanchez 1997). In addition, some *in vitro* experiments have demonstrated increased cytokine production in response to treatment with DEPs. Ohtoshi et al. (1998) used primary nasal and bronchial epithelial cells isolated from human tissues in addition to the BEAS-2B human bronchial epithelial cell line. After exposure to DEPs, all three cell types showed increased production of the cytokines IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF). This was associated with the chemical composition of DEPs and not the carbon particles themselves, since control carbon particles did not induce the effects. Furthermore, they demonstrated a similar increase in cytokine production in BEAS-2B cells exposed to benzo[a]pyrene, an important polycyclic aromatic hydrocarbon found in DEPs. This result suggests that ambient particles that contain low levels of transition metals but higher amounts of organic compounds such as DEPs may induce similar responses in airways of exposed individuals.

Most of the inflammatory effects in the *in vitro* studies described above have also been demonstrated in animal toxicology experiments and have been reviewed elsewhere (Dreher 2000, CEPA/FPAC Working Group 1999, Salvi and Holgate 1999, Martin et al. 1997). Recently, some groups have started to use controlled human exposure trials to study the *in vivo* effects of particles including diesel exhaust particles (Blomberg 2000). For example, an inflammatory response in the airways of healthy human volunteers was observed after exposure to DEPs at concentrations corresponding to high level ambient situations (Nightingale et al. 2000). This response was characterized by increased neutrophils and myeloperoxidase in sputum samples and increased levels of exhaled carbon monoxide, indicating oxidative stress. However, they did not observe differences in TNF-α or IL-8 in the sputum, or in TNF-α or IL-6 in blood samples. Another study monitored the responses of healthy human volunteers to diesel exhaust, which in addition to DEPs contains measurable levels of nitrogen dioxide, nitrogen monoxide, carbon monoxide and formaldehyde (Salvi et al. 2000). Bronchial wash cells and bronchial biopsies were obtained from subjects for analysis. Exposure to diesel exhaust resulted in increased transcription of the IL-8 gene in the wash cells and bronchial epithelium, and increased expression of IL-8 and growth-regulated oncogene-alpha (GRO-α) proteins in the bronchial epithelium. They did not observe changes in levels of TNF-α, IL-6, or GM-CSF, which have been monitored *in vitro*. Nevertheless, the results allow comparisons to be made between *in vitro* studies and actual effects observed in the lungs of human subjects. Further research in this area will help to identify the most relevant biological responses to particulate matter. This will help to strengthen the biological plausibility of the underlying mechanisms and give some insight as to the best *in vitro* systems to use when assessing the effects of PM.

In addition to common inflammatory responses, the oxidative stress induced by exposure to particulate matter may have other mechanisms of action. Changes in the normal immune function of the lungs may be responsible for increased hospital visits and mortality observed in epidemiological studies. Omara et al. (2000) studied the effects of ambient $PM_{2.5}$ collected in Ottawa on rat and mouse splenocytes and rat peripheral blood mononuclear cells isolated from untreated animals. Exposures of these cell populations to PM *in vitro* resulted in decreased proliferation of B and T lymphocytes when challenged by other stimuli. This indicates an impairment in the normal cellular function, which may increase susceptibility of individuals to microbial infections. An increase in production of IL-2 was also reported, indicating the induced inflammatory response seen in other studies. These effects were observed for total, insoluble, and soluble fractions of PM. The inhibition of T and B cell proliferation was reversed by the addition of *N*-acetylcysteine, an antioxidant. This supports the involvement of reactive oxygen species in the toxicological mechanisms of PM.

2.2.2 Genotoxic Effects of PM

Although most of the research on biological mechanisms of particle toxicity has focused on inflammatory responses, other possibilities including genotoxic effects have been investigated. This is important when considering the possibility of an increased risk of lung cancer from long-term exposures to PM, which has been suggested in some epidemiological studies (Dockery et al. 1993). In most cases the chemical species in PM that are known to be genotoxic are organic species, especially polycyclic aromatic hydrocarbons (PAHs). This class of compounds has been extensively studied, and many of them, including benzo[*a*]pyrene, have shown mutagenic or carcinogenic potential. Many other organic compounds present in complex mixtures like diesel exhaust have not been individually tested for mutagenicity. Hornberg et al. (1998) investigated the genotoxicity of ambient $PM_{2.5}$ and PM_{10} by treating a human lung epithelial cell line (BEAS-2B) with organic extracts from particle samples. They observed induction of sister chromatid exchanges, an indicator of genetic damage, with relatively low concentrations of PM. This effect was evident for both size fractions but was particularly prominent for $PM_{2.5}$. The difference in toxicity between fine ($PM_{2.5}$) and coarse ($PM_{2.5-10}$) particulates has also recently been assessed by monitoring cytotoxicity and DNA damage measured by the Comet assay (Hsiao et al. 2000). Solvent extractable organic compounds from PM samples were characterized and used to treat a rat fibroblast cell line. The fraction containing PAHs was found to have the highest toxicity and ability to cause DNA damage. Furthermore, the $PM_{2.5}$ fraction was shown to have a much greater genotoxic potential than the coarse ($PM_{2.5-10}$) fraction. These results are intuitive since organic compounds tend to accumulate at higher levels in the fine fraction of ambient particulate matter. Other groups have also demonstrated the ability of organic compounds in PM samples to generate DNA adducts (Binkova et al. 1998, Dreher 2000). Knaapen et al. (2000) used a slightly different approach looking at DNA damage resulting from particulate-induced oxidative stress. They demonstrated the ability of total suspended particulates (TSP) from Duisburg, Germany to form free radicals in solution. The involvement of transition metals (iron, vanadium and nickel) present in the particles in the oxidative response was identified. Treatment of a rat lung epithelial cell line with extracts from TSP resulted in a two-fold increase of 8-hydroxydeoxyguanosine (8-oxodG), a form of oxidative DNA damage. The formation of this adduct was inhibited by deferoxamine, supporting the involvement of metals in the genotoxicity of the PM sample.

Many of the chemical constituents of ambient particles are capable of inducing a mutagenic or genotoxic response at moderate levels of exposure *in vitro*. Generally speaking, genotoxicity is not considered a main mechanism of particle toxicity in humans due to the lack of convincing epidemiological evidence. One problem is that any increase in lung cancer risk from air pollution is likely to be very small compared to that from smoking or exposure to second-hand smoke, making it very difficult to observe the effects in population studies. Nevertheless, DNA damage and mutation resulting from chronic low-level exposure to particles may contribute to the overall health effects observed, including excess deaths.

2.2.3 Cardiovascular Effects of PM

Respiratory symptoms and exacerbation of preexisting pulmonary disorders are the most obvious health effects from exposure to airborne particulate matter. However, epidemiological findings have also demonstrated an increased risk of cardiovascular problems in populations exposed to high levels of ambient PM (Pope 2000, Samet et al. 2000). The mechanisms involved in cardiovascular effects of PM are not clear, however some theories have been proposed. One suggestion is based on the systemic effect of inflammatory mediators released from epithelial cells or macrophages in the lung. This inflammatory response may cause increased blood coagulability, resulting in increased cardiovascular effects in susceptible individuals (Seaton et al. 1995). This proposal was supported by a study showing increased blood plasma viscosity in men and women exposed to high ambient levels of PM (Peters et al. 1997). A recent preliminary *in vivo* experiment using rats exposed to high levels of ROFA particles resulted in increased levels of fibrinogen in blood samples (Gardner et al. 2000). Fibrinogen levels were not increased when the animals were exposed to Mt. Saint Helen's volcanic ash, a particle sample containing essentially no transition metals. This supported the mechanism of metal-induced release of cytokines or other inflammatory mediators in the changes of plasma fibrinogen. Fibrinogen has been found to increase plasma viscosity and induce aggregation of red blood cells, both of which may be involved in cardiovascular effects in high risk individuals. An increase in plasma fibrinogen was also demonstrated in a controlled human exposure study using concentrated ambient $PM_{2.5}$ collected in downtown Toronto (Petrovic et al. 2000). The response was measured after a period of exercise in healthy human volunteers exposed to $PM_{2.5}$ at levels corresponding to maximum ambient concentrations in Toronto. Although this slight increase of fibrinogen did not result in any measurable symptoms, it provided evidence that effects may be observed in people with existing cardiovascular disease. Mukae et al. (2000) suggested an additional biological effect of PM-induced inflammation that may be involved in cardiovascular responses. They showed that cytokines including TNF-α that were released from alveolar macrophages treated with ambient PM_{10} from Ottawa stimulated processes in bone marrow. This included an increased release of polymorphonuclear cells (macrophages and neutrophils) into the blood circulation, which they suggested may contribute to a systemic response involved in the cardiovascular effects seen in the epidemiology studies. They were unable to identify which component of the PM sample was associated with the observed effects, but indicated that endotoxin did not play an important role.

2.2.4 Summary

Analysis of the available literature on the biological effects of PM does not suggest a clear mechanism of toxicity. Inflammatory responses including increased release of cytokines and other mediators is the most likely cause of the respiratory effects seen in population studies of PM exposure. This mechanism may involve generation of reactive oxygen species by different chemical constituents of particulate matter. Experiments addressing the issue of PM-induced cardiovascular effects have suggested that, like respiratory responses, an inflammatory mechanism may be involved. Genotoxicity may also be an important effect of chronic exposure to PM when considering the possibility of an increased risk of lung cancer. Depending on the particulate samples used and the responses being monitored, a variety of components of PM have been implicated in its biological effects. These include iron, copper, vanadium, other transition metals, polycyclic aromatic hydrocarbons and other organic species, endotoxins, and the ultrafine fraction of $PM_{2.5}$ (summarized in Table 1). The ability to define the most important components involved in PM toxicity is hindered by the complex composition of ambient PM samples. This is further complicated by variability in PM characteristics dependent on major pollution sources, geographic location, and seasonal changes. In a real-life exposure situation, it is likely that a combination of these chemicals is responsible for health effects in humans. However, with continued research it may be possible to achieve a consensus identifying the components that are most harmful to human health.

Table 1. **Summary of chemical constituents associated with the biological effects of particulate matter. Some studies identified constituents that were not likely to be associated with the observed effects.**

REFERENCE	BIOLOGICAL RESPONSE	IMPORTANT COMPONENTS OF PARTICULATE	NON-ASSOCIATED COMPONENTS
Li et al. 1997	TNF-α and NO increase	oxidants	
Kennedy et al. 1998	IL-6, IL-8 levels, NF-κB activity	copper, soluble fraction	iron, lead, zinc
Carter et al. 1997	IL-6, IL-8, TNF-α levels / mRNA	vanadium	iron, nickel
Frampton et al. 1999	IL-6, IL-8 levels	transition metals	endotoxin
Jimenez et al. 2000	NF-κB activity	iron	endotoxin
Salonen et al. 2000	IL-6, NO levels	iron	
Imrich et al. 2000	TNF-α, MIP-2, NO levels	endotoxin, insoluble	copper, iron
Ning et al. 2000	TNF-α, MIP-2 levels	endotoxin	
Shukla et al. 2000	NF-κB activity	ultrafine fraction	iron
Ohtoshi et al. 1998	IL-8, GM-CSF levels	organics, PAHs	carbon particles
Hornberg et al. 1998	genotoxicity	organics, $PM_{2.5} > PM_{10}$	
Hsiao et al. 2000	DNA damage	PAHs, $PM_{2.5} > PM_{2.5-10}$	
Knaapen et al. 2000	oxidative DNA damage	transition metals	
Gardner et al. 2000	plasma fibrinogen levels	transition metals	

3.0 COMMON PROTOCOLS USED IN PM RESEARCH

Since there are a variety of methods used to assess the biological effects of particulate matter, it is not possible to describe each individually in detail. However, there are relatively common procedures that are used for collection, analysis, and preparation of PM prior to treatment. Cell treatment protocols will be presented for *in vitro* experiments, and will include general techniques for maintaining cell lines in culture as well as typical exposure parameters. This will be followed by descriptions of some of the more common biological responses that have been studied. Since these protocols are based on the recent publications reviewed in this document, they should be fairly representative of the most current methods in use. In most cases companies that are listed as suppliers of products used in these methods are not the sole supplier, but were referenced in the reviewed articles and are included as examples.

3.1 Collection and Preparation of Ambient Particulate Matter

Particles from ambient air are typically collected onto either Teflon or glass fiber filters using a variety of high-volume air samplers with inlets and cutoff stages for PM_{10} or $PM_{2.5}$. The time of sample collection varies, but a 24 hr period is common. Particulates are recovered by suspending the filters in solution (e.g. phosphate buffered saline (PBS), 0.9% saline, or water for samples to be analyzed by ICP-MS), followed by vortexing and either bath or probe sonication. The duration of ultrasonic treatment ranges from a few minutes to one hour, and serves to dislodge the particles from the filters and create an even suspension. Alternatively, the particles may be extracted with a solvent (H_2O, dichloromethane, methanol) specific for the chemical components of interest. In each case insoluble particles may be separated from the supernatant/extract by centrifugation (13,000*g* for 10 min). For aqueous procedures, the soluble and insoluble fractions are then lyophilized or air-dried and weighed using a microbalance to estimate concentration. The particles or lyophilized supernatant are then resuspended or dissolved for analysis and cell treatments, and may be stored at –70°C until use. Organic extracts are usually air-dried and redissolved in dimethylsulfoxide (DMSO) for *in vitro* treatments.

3.2 Physical and Chemical Characterization of PM

Ambient PM samples are usually characterized to determine the size range of particles and their chemical composition. Size distribution may be determined by a variety of techniques including flow cytometry and scanning / transmission electron microscopy (STEM). Chemical analysis methods depend on the constituents being measured. Inorganic species including metals are typically determined by inductively coupled plasma – mass spectrometry (ICP-MS), ICP-emission spectroscopy, (ICP-ES), or energy dispersive x-ray analysis. Ions present in the particle matrix may also be analyzed by ion chromatography. Organic compounds extracted from PM samples are characterized by high performance liquid chromatography (HPLC) or gas chromatography (GC) systems combined with MS or fluorescence detection. Levels of endotoxin in particle samples are typically measured using commercially available *Limulus* chromogenic assay kits (Sigma, BioWhittaker). In addition to these component analyses, particles may be tested for their overall ability to generate reactive oxygen species. This is usually accomplished using the Fenton reaction; when oxidants from PM are mixed with deoxyribose, the resulting product reacts with thiobarbituric acid (TBA) to form a pink chromophore which is measured with a spectrophotometer (Aruoma 1994). This procedure may include the addition of deferoxamine (1 mM) to assess the contribution of transition metals to the oxidant potential.

3.3 Cell Culture

Techniques used for cell culture in PM experiments are essentially standard procedures. Cells are typically grown at 37°C in incubators with a humidified atmosphere containing 5% CO_2. Cells are grown on culture plates to 80-100% confluence in a variety of media prior to treatment. The culture medium is often specific to the cell type being used, and may include the following:

- keratinocyte growth medium (KGM, Clonetics) for BEAS-2B epithelial cell line
- bronchoepithelial growth medium (BEGM, Clonetics) for primary isolated epithelial cells
- hormonally defined Ham's F12 medium for BEAS-2B or primary epithelial cells
- Dulbecco's Modified Eagle's Medium (DMEM, Sigma Life Technologies) with 10% fetal bovine serum and antibiotics for A549 lung epithelial cell line
- RPMI 1640 medium (BioWhittaker) with 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics for alveolar macrophages

3.4 *In Vitro* Treatment of Cells with PM

Prior to cell treatment, stock solutions (1 mg/ml) and appropriate dilutions of ambient particles or supernatants are typically prepared in the medium being used for cell culture. Alternatively, particles may be suspended in a buffer solution (PBS) with or without deferoxamine (2 mM) for 16 hr prior to treatment to assess the effect of metals on the exposed cells. Dilutions of PM in medium are then added to cell cultures and incubated at 37°C, 5% CO_2 for a period of time before measuring the biological responses. Doses of PM (or equivalent doses of supernatant) used are typically between 20-100 μg/ml final concentration, with doses as high as 500 μg/ml used to determine cytotoxicity of particles. Common treatment incubation times are 2 and 24 hrs, although intermediate durations have also been used. Deferoxamine (1 mM) may be added to the culture medium during the exposure period. After exposure, cell supernatant may be collected and stored at –80°C for cytokine assays or cells may be washed with PBS prior to further analysis, depending on the biological response being studied.

3.5 Biological Responses

3.5.1 Cell Viability

The viability of cells used in these experiments must be determined for two reasons. First, cells that are isolated from human or animal tissues (primary epithelial cells or macrophages) must be proven to be viable prior to *in vitro* exposure. Regardless of the cell type used, cell viability must also be measured to evaluate the cytotoxic effects of particulate matter. This is important because responses such as cytokine production may be influenced by cell death in the treated sample, particularly at high doses of PM. Cell viability is commonly determined by one of three procedures. One method is to measure the release of lactate dehydrogenase (LDH) into the cell supernatant, which is an indication of a loss of membrane integrity. LDH assay kits are commercially available (Sigma). Another common colorimetric method is the MTT assay, which detects functioning mitochondria (Mosmann 1983). Both these techniques are relatively simple and do not require specialized equipment. A third method for cell viability uses propidium iodide, a red fluorescent DNA-binding dye that indicates membrane permeability. Cells stained with propidium iodide are then analyzed using a flow cytometer.

3.5.2 Cytokine and Nitric Oxide Production

Cytokines and nitric oxide are inflammatory mediators that are released by cells exposed to ambient particles. Supernatant from treated cells is frozen (-20/-80°C) prior to cytokine determination. Enzyme-linked immunosorbent assay (ELISA) kits are commonly used and are commercially available for a range of cytokines including IL-2 (Endogen), IL-6 (R&D Systems, Pharmingen), IL-8, TNF-α, and GM-CSF (R&D Systems). Nitric oxide is typically measured as the stable metabolite nitrite using the Griess spectrophotometric method (Green et al. 1982).

3.5.3 Gene Expression

Treatment of cells with ambient particulate matter *in vitro* may induce expression of a variety of genes involved in the inflammatory response. This results in increased levels of messenger RNA (mRNA) encoding proteins such as the cytokines IL-6 and IL-8. PM-induced increases of cytokine mRNAs have been quantified using the reverse transcriptase – polymerase chain reaction (RT-PCR) technique. Cells are washed in PBS then lysed and RNA is isolated by ultracentrifugation through cesium chloride. Total mRNA is then converted to cDNA with reverse transcriptase enzyme. cDNA corresponding to the genes of interest is preferentially amplified using a thermocycler and oligonucleotide primer pairs with gene-specific sequences. The resulting products are separated by agarose gel electrophoresis, stained and photographed with ultraviolet illumination. The bands are then analyzed with a densitometer or other imaging device to quantify relative gene expression.

3.5.4 NF-κB Activity

The DNA-binding protein NF-κB promotes expression of genes involved in the inflammatory response. Differences in the levels and activity of NF-κB in the nuclei of cells exposed to PM can be monitored using fluorescence microscopy or electrophoretic mobility shift assays. Primary antibodies against NF-κB are available, and can be used with fluorescent secondary antibodies to visualize the protein in cells with a fluorescence microscope. Mobility shift assays are required to quantify DNA-binding activity of NF-κB. After treatment, cells are washed then lysed and nuclei are collected by centrifugation. Nuclear protein extracts are incubated with ^{32}P-radiolabelled double-stranded oligonucleotides containing the NF-κB binding sequence for 20 minutes at room temperature. The complexes are then separated using polyacrylamide gel electrophoresis under native conditions and visualized by exposing x-ray films to the radioactivity or by using a phosphorimaging system.

4.0 EXPERTISE AND EQUIPMENT REQUIREMENTS

Any research combining chemical characterization of particulate matter with determination of its biological effects would require a variety of equipment and skills. Broadly speaking, experience is necessary in the following disciplines:

1. analytical chemistry;
2. cell culture;
3. cell biology / biochemistry / molecular biology

While skills in each of these areas would be an asset, perhaps the most crucial requirement would be considerable experience in cell culture and handling techniques. This would further include skilled animal technicians if experiments were to be performed on cells harvested from either untreated or treated animals. Cell culture itself is a highly specialized and delicate technique, with success being based largely on first-hand experience. Training in biosafety and sterile techniques would be a minimum requirement for personnel doing cell culture. A background in analytical chemistry would also be necessary for chemical characterization of PM, since the instruments used for these determinations require experienced operators. Finally, experience in biochemical and molecular biology techniques including gel electrophoresis, immunoassays, PCR and flow cytometry would be beneficial depending on the biological effect under investigation. Some of the biological techniques may involve working with radioisotopes, therefore lab personnel would also require radiation safety training.

Depending on the experimental approach, laboratory facilities or major pieces of scientific equipment required for this type of research may include:

- air particulate sampling apparatus with PM_{10} and/or $PM_{2.5}$ cutoffs
- ultrasonic water bath and/or ultrasonic probe
- microbalance
- freeze dryer
- ultra-low temperature freezer (-80°C)
- microcentrifuge
- ultracentrifuge
- scanning / transmission electron microscope (for particle analysis or biological responses)
- analytical equipment
    - ICP-MS or ICP-ES for metal / elemental content
    - GC-MS, HPLC-MS or HPLC-fluorescence detection for organic content (including PAHs)
- incubators with temperature, humidity and CO_2 control
- biosafety cabinets and flow benches
- flow cytometer (for particle analysis, cell viability and monitoring biological responses)
- light or fluorescence microscope
- microplate reader / spectrophotometer (visible or fluorescent)
- conventional spectrophotometer
- polyacrylamide and/or agarose gel electrophoresis apparatus and power supply

- imaging equipment (densitometer or phosphorimager)
- ultraviolet transilluminator
- thermocycler for PCR
- water baths, heating blocks, rotary mixers, and other common laboratory equipment

Methods for determining biological responses that differ from those found in the reviewed literature may require additional equipment. The research laboratory facilitating this work would require biosafety and possibly radioactivity certification. Furthermore, if cells used for experimentation are to be isolated from treated or untreated animals, access to a complete animal care facility would be necessary.

5.0 CONCLUSION

Despite numerous studies being performed, there is still debate as to the level of risk that exposure to airborne particulate matter represents to human populations. Criticisms of the causal inference between PM and health effects in humans include the lack of both uniform analytical methods used in epidemiological studies and a biologically plausible mechanism of particle toxicity. A recent review of epidemiological data and air pollution measurements for 20 major U.S. cities used a more rigid analysis and reinforced the association between ambient PM_{10} levels and excess mortality from respiratory and cardiovascular illnesses (Samet et al. 2000). The study also concluded that co-pollutants found associated with PM including carbon monoxide, nitrogen dioxide, and sulfur dioxide were not related to the excess deaths. Laboratory-based studies have demonstrated a number of biological responses that may represent plausible mechanisms for PM-induced health effects in humans. However, the most important chemical species in particulate matter relative to these responses has not been confirmed. It is likely that the most toxic component of PM would vary depending on the pollution sources in the environment.

One argument against the implementation of revised PM standards in the U.S. is that the cost to industry associated with reduction of ambient $PM_{2.5}$ levels would greatly outweigh any benefits to human health (Ware 2000). Because of the lack of conclusive data about the most toxic constituents of PM, current regulations are based on particle mass. It is thought that if the most important species are identified, then PM standards could be revised to include size or chemical definitions. This would focus the regulatory efforts on the most important sources of particulate pollution, increasing the benefit to public health while at the same time moderating the effect on the economy. The chemical profile of particles emitted from different sources can vary significantly. This fact is used to model the contribution of multiple pollution sources to the overall mixture of particles in the atmosphere, a technique called source apportionment. A recent study assessed the relationship between excess deaths and chemical composition of ambient PM by combining epidemiological data with chemical speciation and source apportionment modeling using factor analysis (Tsai et al. 2000). The study demonstrated significant associations between mortality and derived sources including oil burning, industry, sulfate aerosol, and motor vehicles.

Further advances in identifying the most relevant pollution sources are dependent on the elucidation of the most important mechanisms of particle toxicity and the associated chemical components of PM. However, the ability to screen particulate samples from various sources depends on the development of a reliable assay. Numerous *in vivo* and *in vitro* biological systems have been used in this area of research. Animal models, while being able to provide valuable information on the effects of PM, are prohibitively expensive for routine or large-scale screening of environmental samples. Therefore, an *in vitro* cellular assay based on the most relevant biochemical responses to PM would be desirable. The advantage of such a technique would be relatively low cost and time of analysis. The resulting data would likely strengthen the observations from the epidemiological studies and provide a clearer picture of the relationship between airborne PM and health effects in humans. For these reasons, continued research on the biological effects of particulate matter is necessary and worthwhile.

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7.0 GLOSSARY OF TERMS AND ACRONYMS

8-oxodG	8-hydroxyguanosine, a type of oxidative DNA damage
Alveolar	pertaining to the alveoli, the small sacs in the deepest part of the lungs where gas exchange with the blood supply occurs
BAL	bronchoalveolar lavage, a technique for recovering cells from the respiratory tract of experimental animals
BEAS-2B	human lung epithelial cell line
BEGM	bronchoepithelial growth medium
CCME	Canadian Council of Ministers of the Environment
cDNA	complementary deoxyribonucleic acid, it is copied from cellular mRNA by reverse transcriptase enzyme
CEPA/FPAC	Canadian Environmental Protection Act/Federal-Provincial Advisory Committee
COPD	chronic obstructive pulmonary disease
Coarse PM	commonly refers to the fraction of particulate matter with an aerodynamic diameter of less than 10 μm but greater than 2.5 μm
CWS	Canada-Wide Standard
Cytokine	one of a group of small proteins that serve as messengers in inflammatory and immune responses, often by stimulating macrophages and other cells
Deferoxamine	chelates (binds) transition metals
DEPs	diesel exhaust particles
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethylsulfoxide, an organic solvent
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay

Endotoxin	also known as lipopolysaccharide, orginates from the cell walls of gram negative bacteria
Epithelial	refers to the layer of cells that forms the lining of tissues such as lung or intestine
Ferrozine	specifically chelates (binds) iron in the +2 oxidation state (Fe^{2+})
Fine PM	the fraction of particulate matter with an aerodynamic diameter of less than 2.5 µm, also known as $PM_{2.5}$
GC	gas chromatography
GC-MS	gas chromatography – mass spectrometry
Genotoxic	referring to anything that causes damage to DNA resulting in genetic abnormalities within a cell
GM-CSF	granulocyte-macrophage colony-stimulating factor, a cytokine
GRO-α	growth-regulated oncogene-alpha, a cytokine
GSH	glutathione, an antioxidant molecule found in lung and other tissues
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography - mass spectrometry
ICP-ES	inductively coupled plasma emission spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry
IgE	immunoglobulin E
IL-2	interleukin-2, a cytokine
IL-6	interleukin-6, a cytokine
IL-8	interleukin-8, a cytokine
KGM	keratinocyte growth medium
LDH	lactate dehydrogenase, an enzyme that may be measured to indicate cell membrane leakage

Lyophilized	freeze-dried
Macrophage	a cell involved in immune responses, its functions include phagocytosis of foreign particles and release of cytokines to stimulate other cells
Mediator	a molecule that is involved with or stimulates a biological pathway or response
MIP-2	macrophage inflammatory protein, a cytokine
mRNA	messenger ribonucleic acid, encodes for the production of proteins in a cell
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a reagent used in a colorimetric assay for functioning mitochondria. The dye is cleaved to colored product by the activity of dehydrogenase enzymes and this indicates high levels of mitochondrial activity in cells.
Neutrophil	a cell that phagocytoses and destroys foreign particles, it is involved in the inflammatory response
NO	nitric oxide, an inflammatory mediator
NF-κB	nuclear transcription factor, enhances the expression of some genes involved in the inflammatory response by binding to specific sequences within the genes
Oligonucleotide	a short segment of DNA typically designed with a specific sequence
Oxidant	see ROS
PAHs	polycyclic aromatic hydrocarbons, byproducts of combustion, many of which have mutagenic or carcinogenic potential
PBS	phosphate buffered saline
PCR	polymerase chain reaction, a technique used to amplify specific sequences of DNA
Phagocytosis	a process by which cells surround and internalize foreign particles
PM	particulate matter

$PM_{2.5}$	particulate matter with an aerodynamic diameter of less than 2.5 μm
PM_{10}	particulate matter with an aerodynamic diameter of less than 10 μm
PMN	polymorphonuclear, refers to white blood cells (macrophages and neutrophils) that are involved in inflammatory or immune responses
RNA	ribonucleic acid
ROFA	residual oil fly ash, a type of particulate matter emitted by industries that burn heavy oil
ROS	reactive oxygen species, also known as oxidants, may cause oxidative damage to tissues and/or biomolecules such as DNA or protein
RPMI	Roswell Park Memorial Institute culture medium
RT-PCR	reverse transcriptase – polymerase chain reaction, a method used to quantify levels of specific messenger RNA present in cell extracts
STEM	scanning / transmission electron microscopy
TBA	thiobarbituric acid
TNF-α	tumor necrosis factor alpha, a cytokine
Transcription	the process of copying a DNA sequence (i.e. a gene) into mRNA
Translocation	movement of a molecule across a cell membrane, i.e. from the cytosol into the nucleus
TSP	total suspended particulates, the entire amount of particles present in ambient air samples, including PM_{10} and much larger particles
Ultrafine PM	particulate matter with an aerodynamic diameter of less than 0.1 μm
US EPA	United States Environmental Protection Agency

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